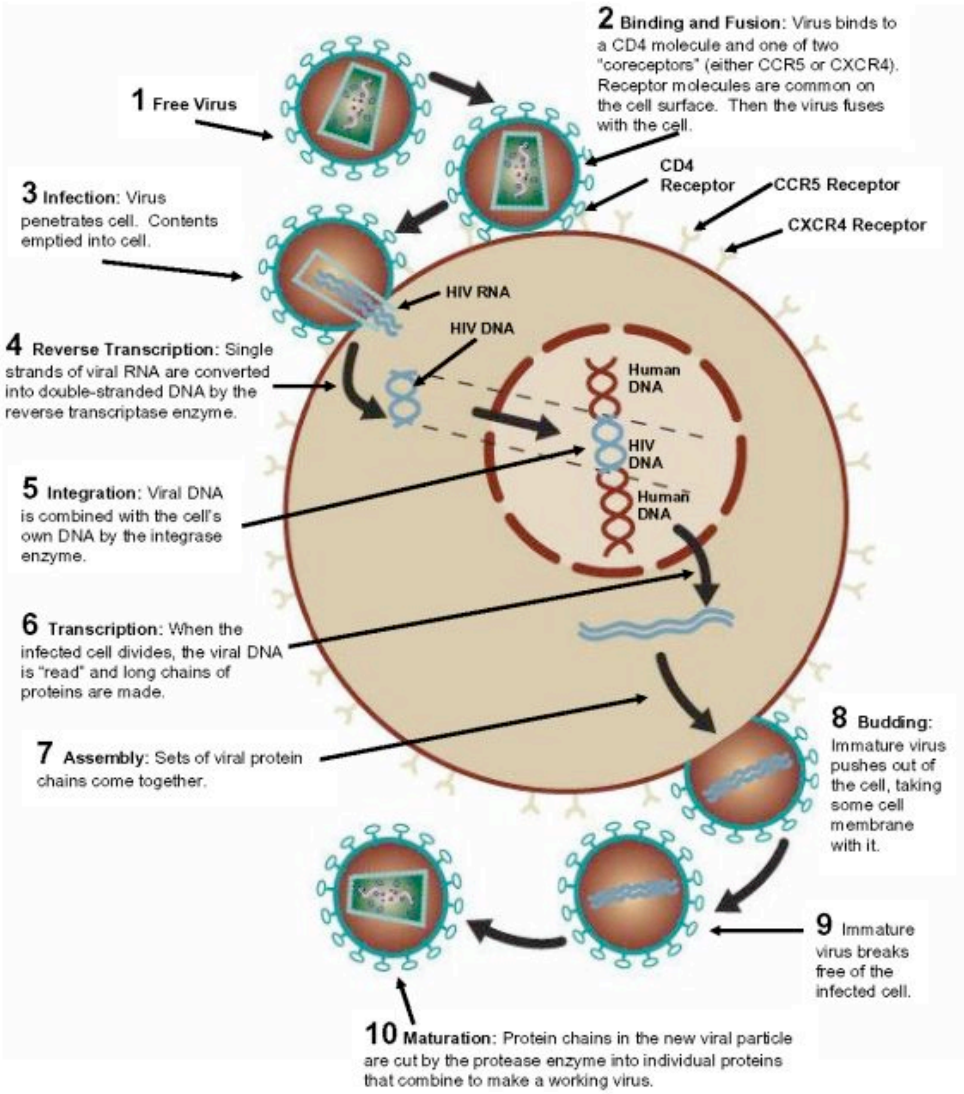
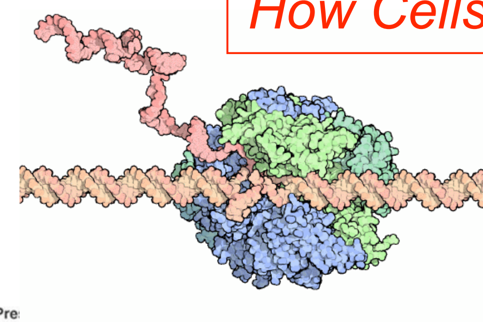
Lecture 4: Binding in Biology: A View From Statistical Mechanics

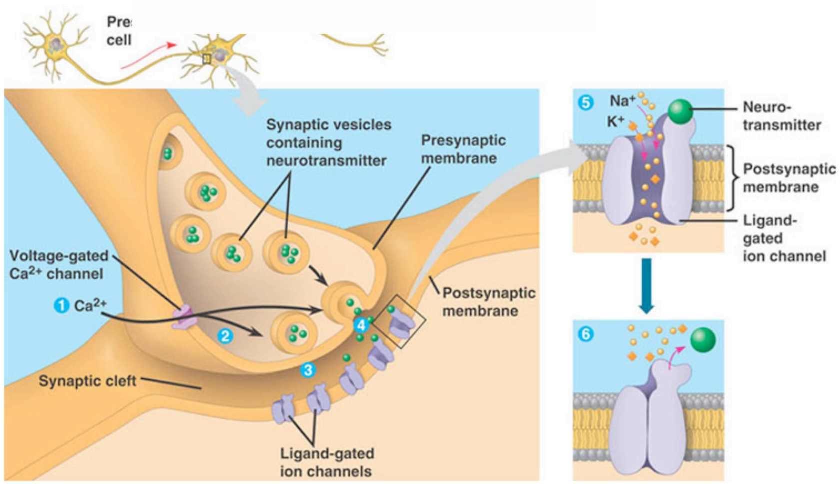


The Biological Importance of Binding

- *``Corpora non agunt nisi ligata'' - ``A substance is not effective unless it is linked to another.'' - Paul Ehrlich*
- *Biology is built around molecular recognition!*

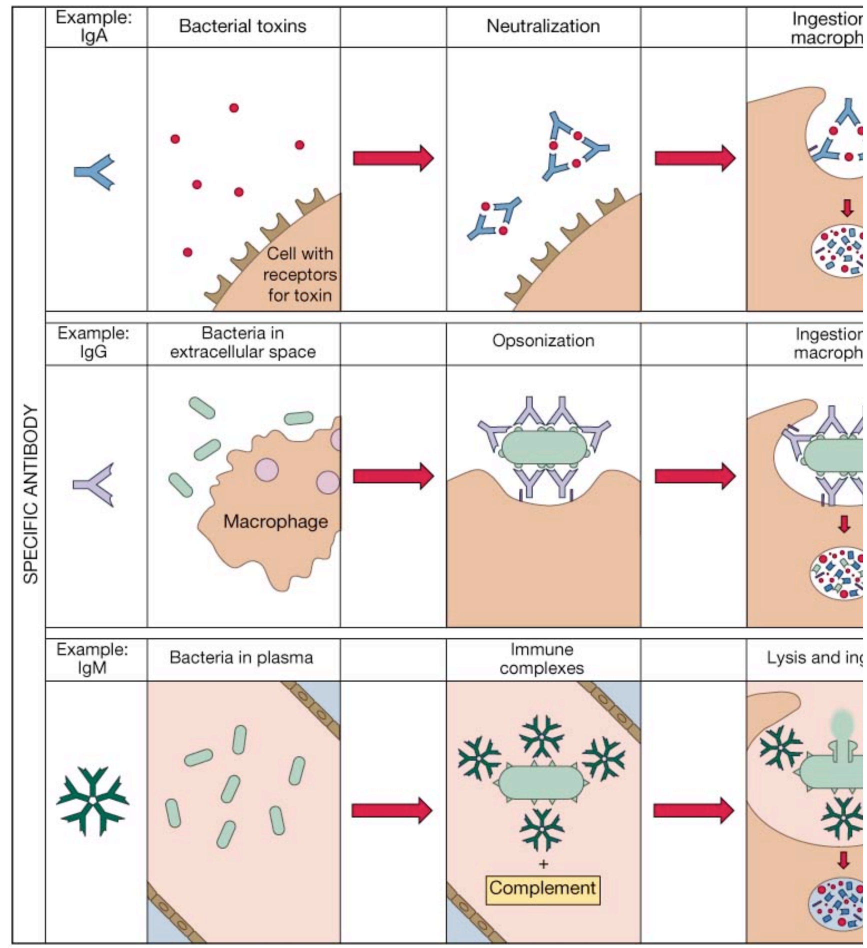
How Cells Decide





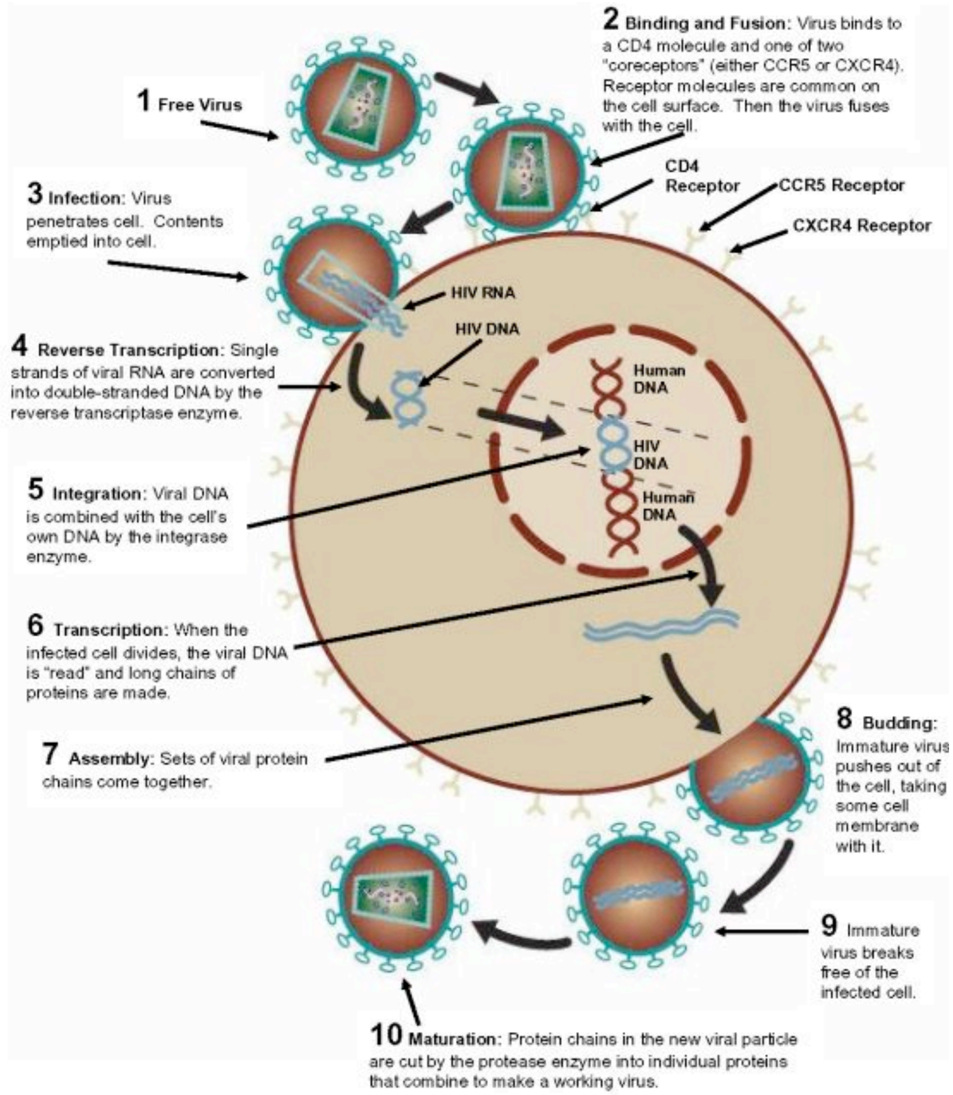
Ligand Gated Channels

The Immune System



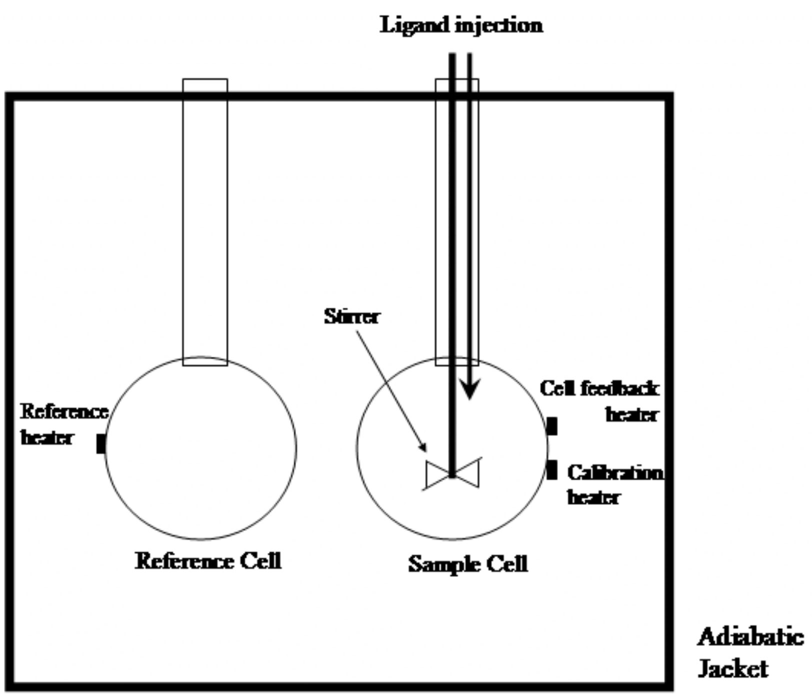
The Role of Binding in the HIV Life Cycle

- *Key point: One of the first processes in the infection process is the binding of the virus to the host cell.*
- *One class of measurements that characterize binding processes like this is the measurement of the ``affinity''.*
- *From a conceptual point of view, we might want to know how other competitor molecules bind to the HIV gp120 in comparison with the CD4 receptor.*

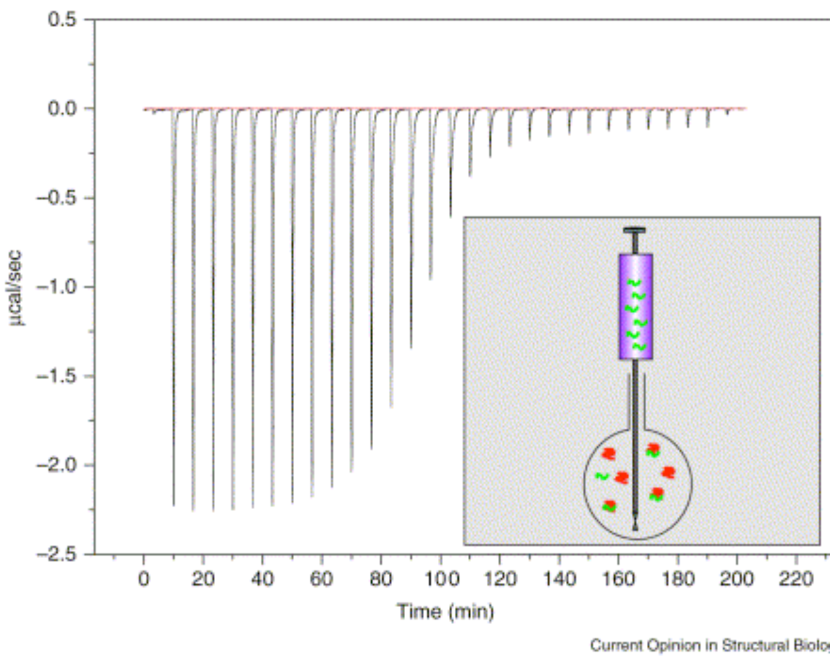


How Are Measurements Made?

Isothermal Titration Calorimetry



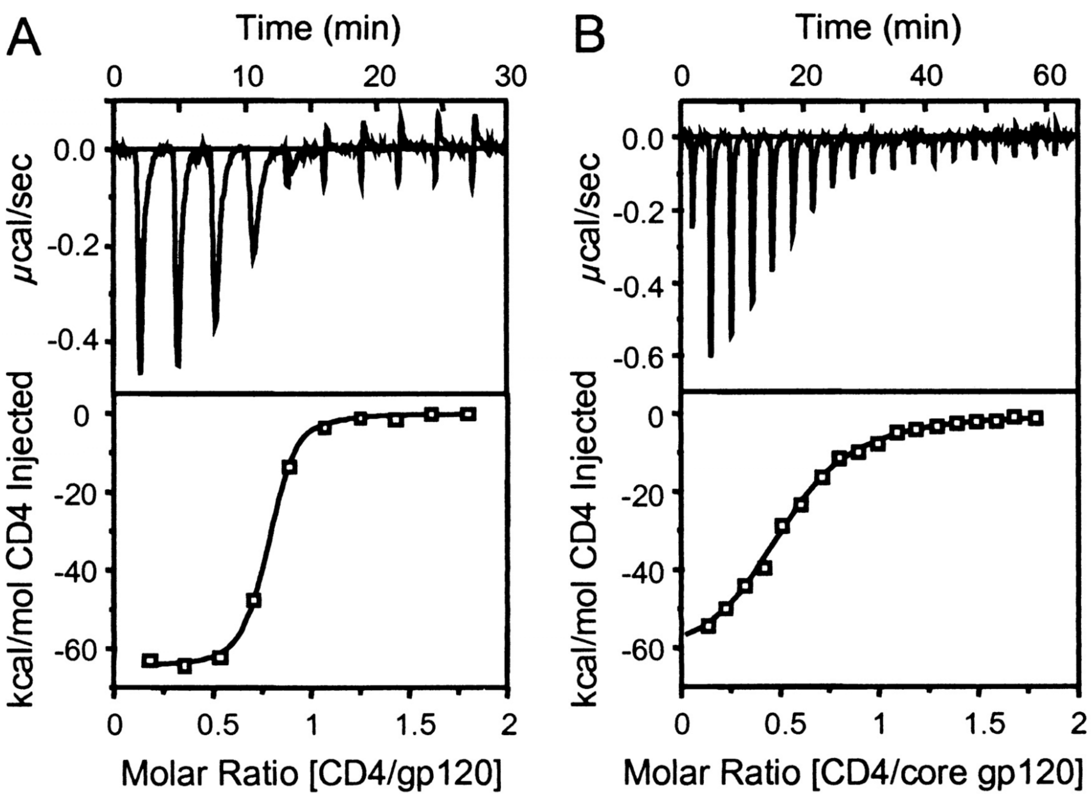
Leavitt and Freire, 2001

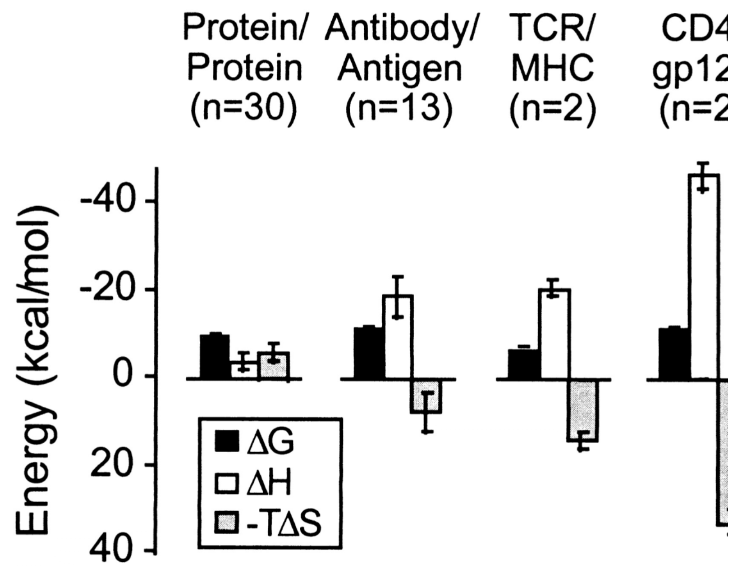


- *Measure the heat released during binding reaction.*
- *Done by comparing how much energy needed to keep the temperature constant in the reactive chamber and in a reference chamber.*

What Is the Data Like? A Case Study from HIV

CD4-gp120 thermodynamics. Calorimetry data for the titration of WD61 full-length (A) and core (B) gp120 with CD4 in 10 mM Na2HPO4, 200 mM NaCl, and 0.5 mM EDTA (pH 7.4). The top panels show raw data in power versus time. The area under each spike is proportional to the heat produced at each injection. The lower panels show integrated areas normalized to the number of moles of CD4 injected at each injection step. Best-fit curves represent binding enthalpy changes of -63 and -62 kcal/mol CD4 for full-length and core gp120, respectively. Equilibrium binding KD values were determined as 5 nM and 190 nM, respectively.





Hendrickson et al.

A Second Set of Binding Problems: Antibody-Antigen Binding

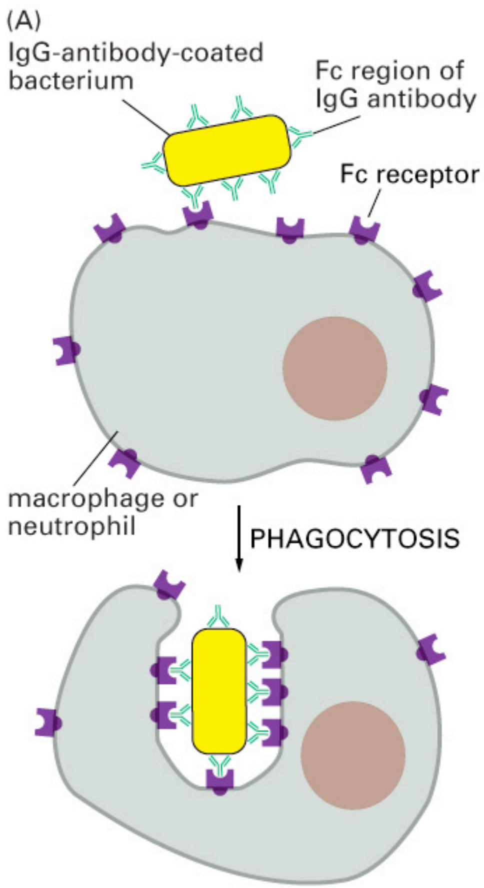


Figure 24–24 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

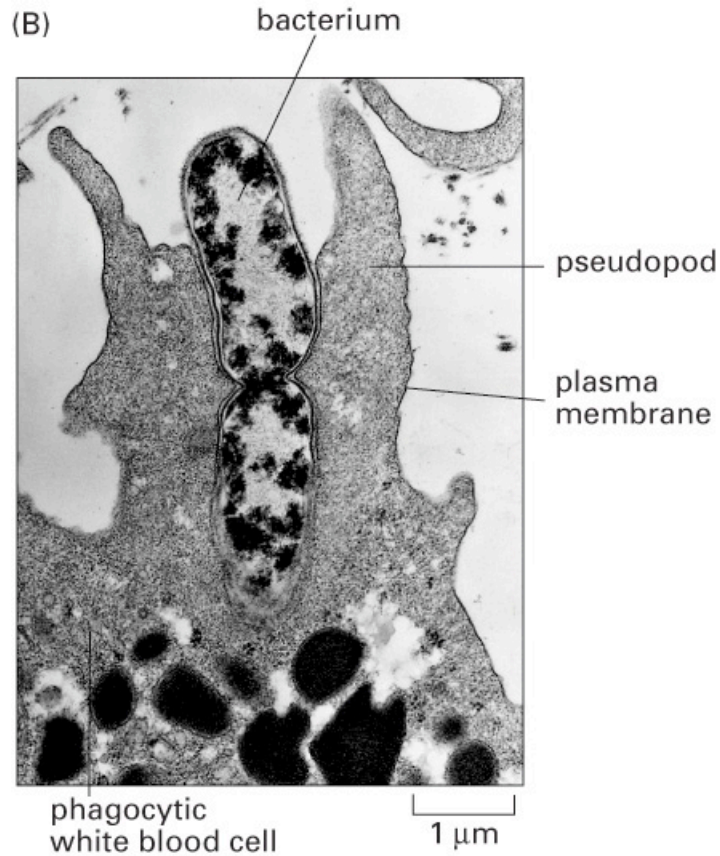


Figure 24–24 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

- *Molecular recognition mediated by binding reactions is at the center of immune response.*
- *Macrophage devour labeled (covered with antibodies) invaders.*

A Primer on Antibodies

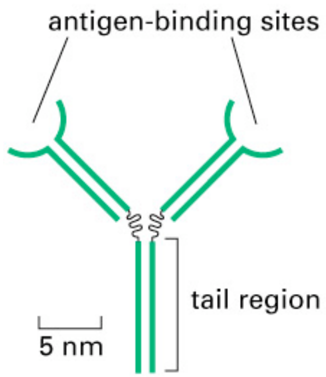


Figure 24–18. Molecular Biology of the Cell, 4th Edition.

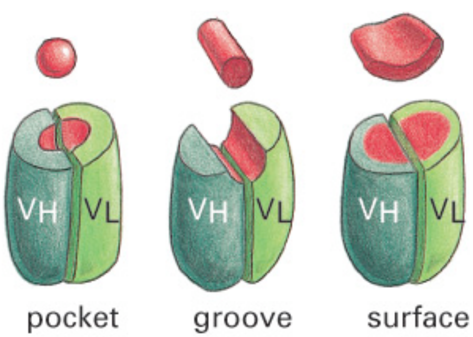


Figure 24–35. Molecular Biology of the Cell, 4th Edition.

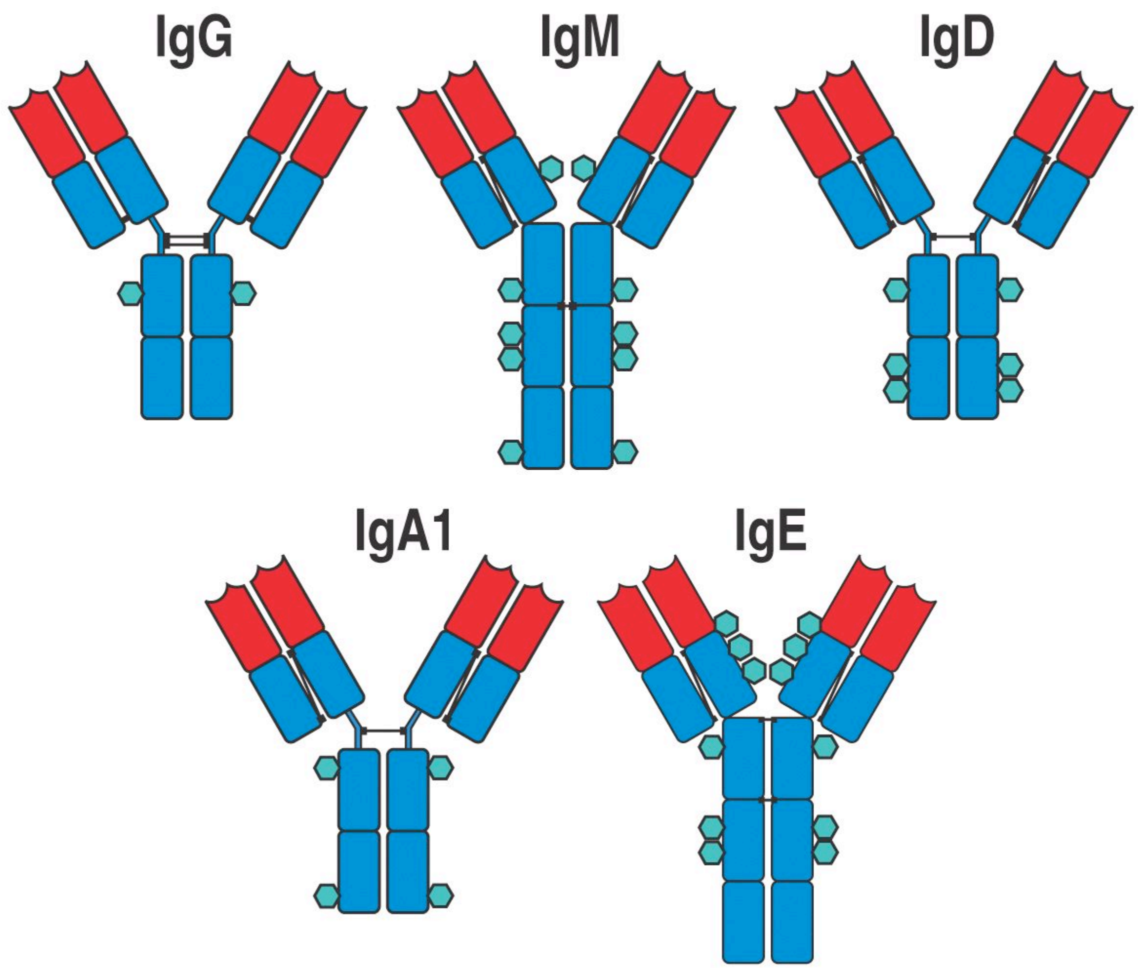


Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

- *Antibodies are the molecular sentinels.*
- *Later we will attack the fascinating question in genome management*

Binding by Neutralizing Antibodies

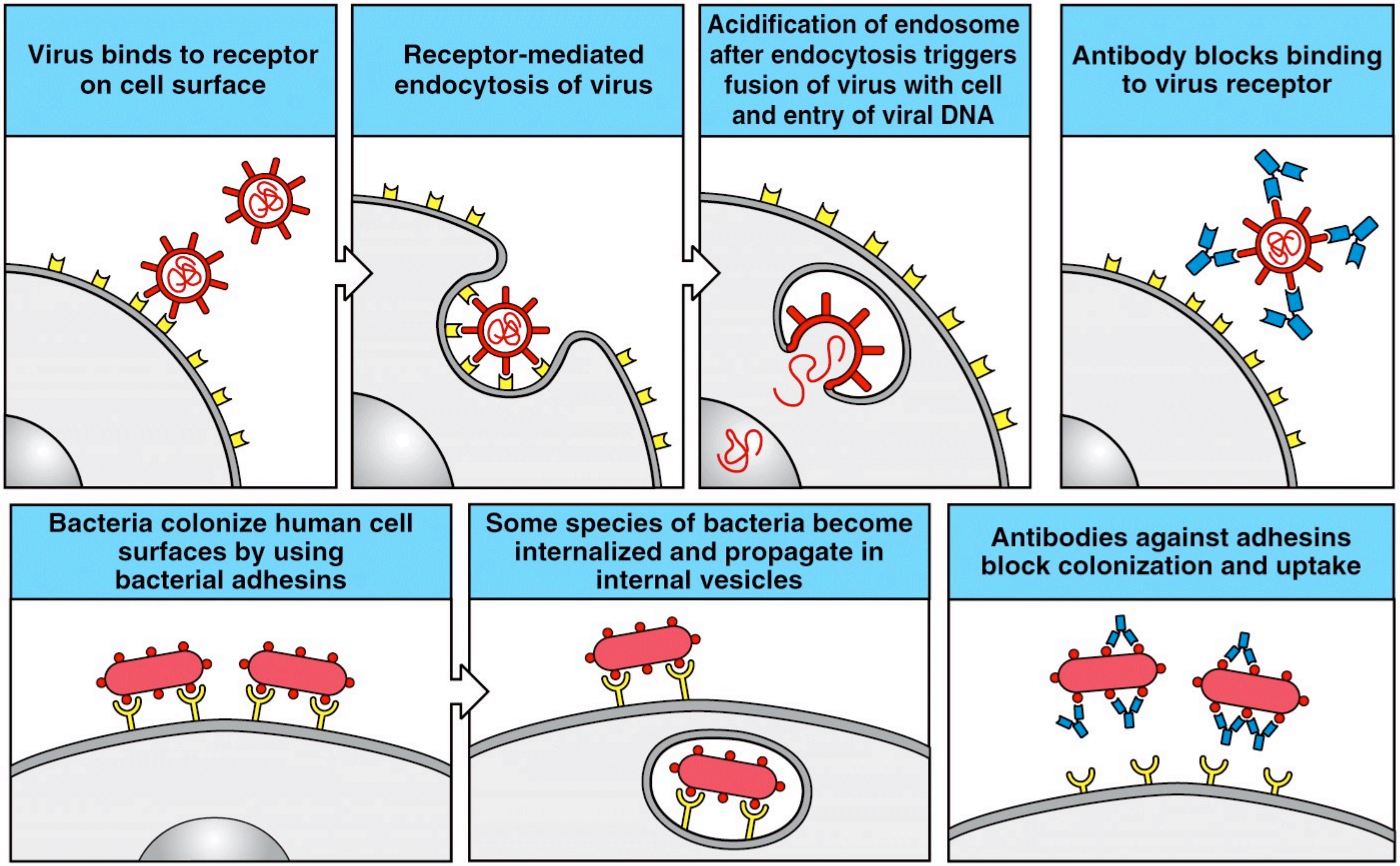
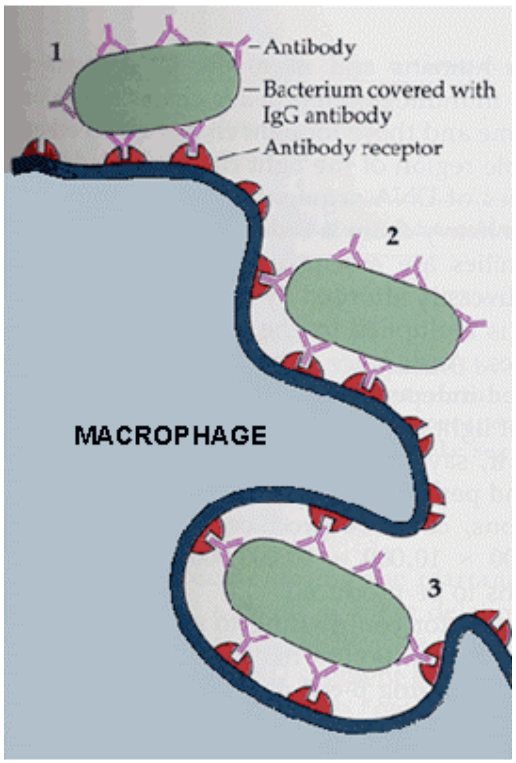
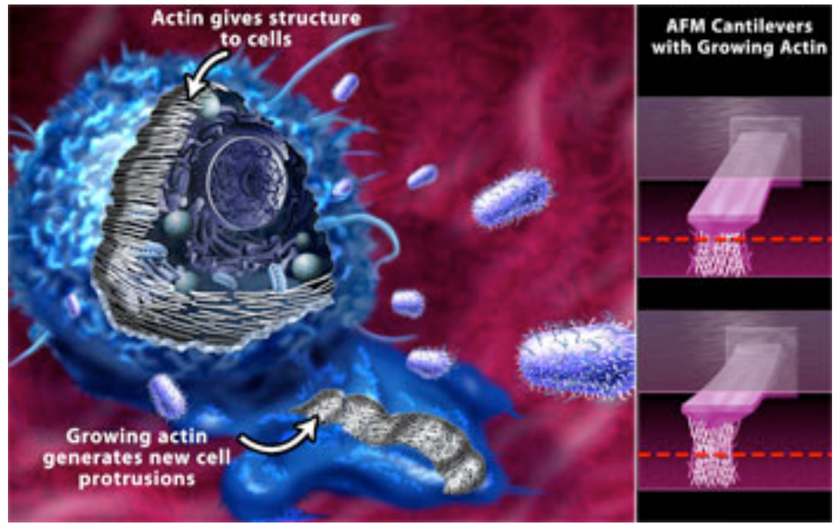
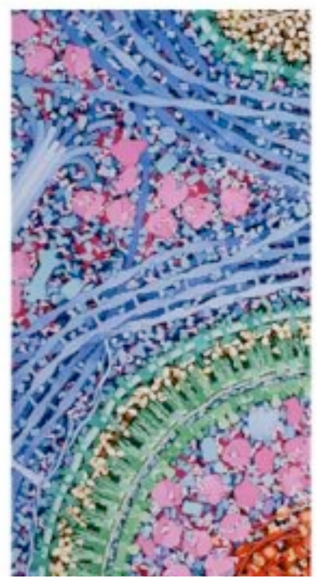


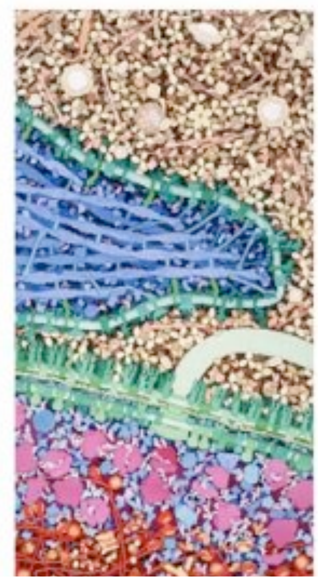
Figure 7-23 The Immune System, 2/e (© Garland Science 2005)

How the Invader Is Destroyed





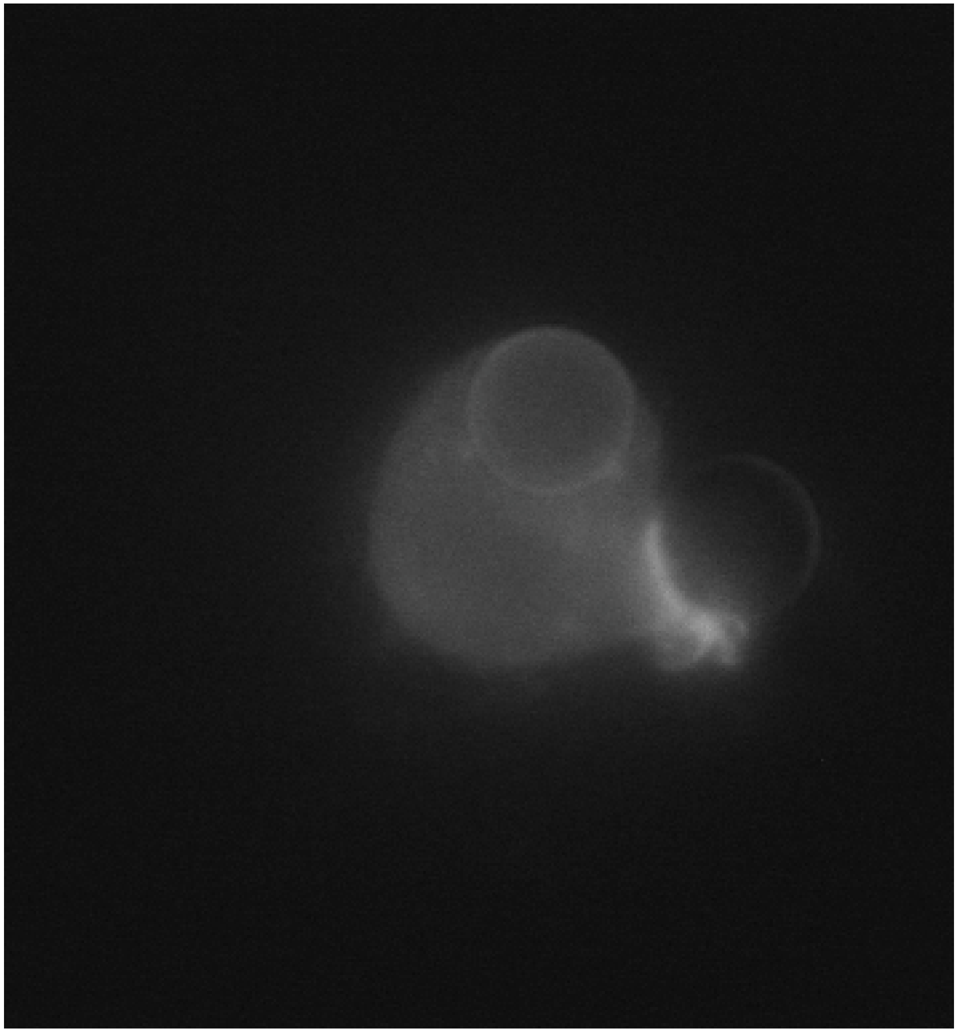




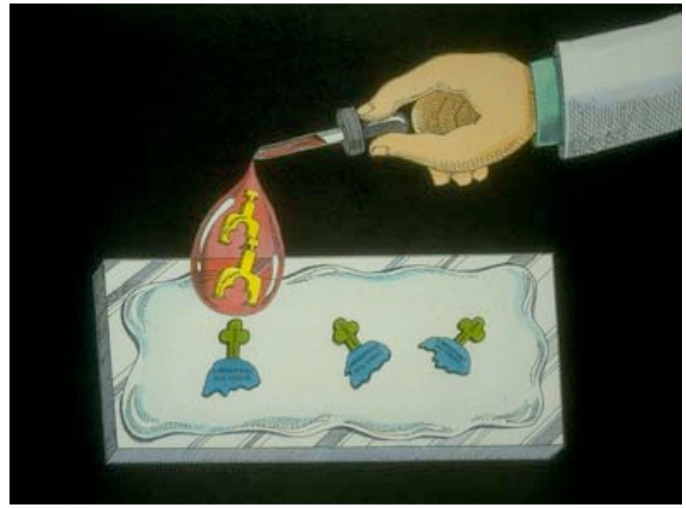
Macrophage Performing Phagocytosis: Recognition Mediated by Antibodies

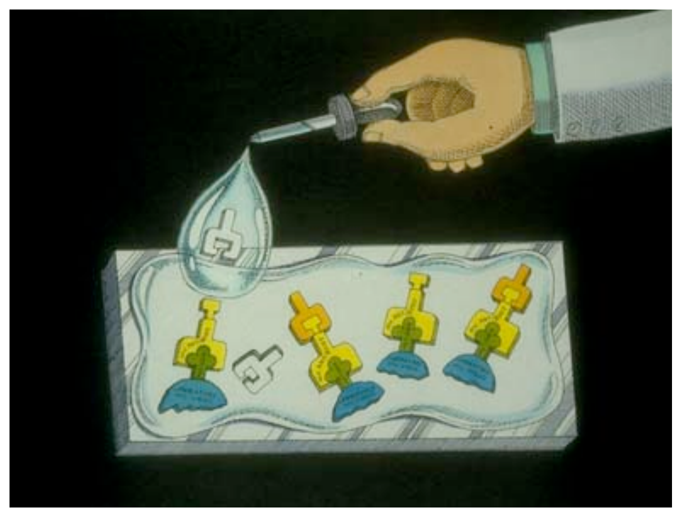
- *Antibodies glued to 5 micron beads.*
- *Macrophage engulfs the bead.*

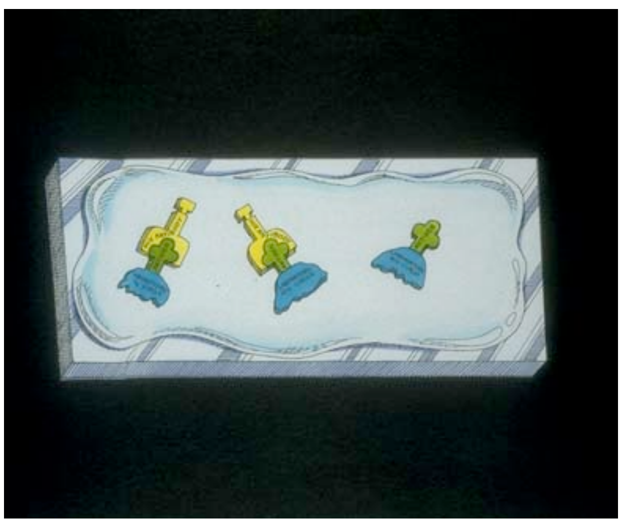
You can see fluorescent actin being recruited to (and presumably polymerized near) a 5 micron anti-body coated bead. The 1st step is the formation of a "phagosomal actin cup". That's the fluorescent blob at the base of the 5micron bead. The actin front then works around the perimeter of the bead, to pinch the membrane closed at the opposite end. After the bead is engulfed, the actin filaments then de-polymerize and redistributes through out the cell.



A Reminder on Elisa and HIV

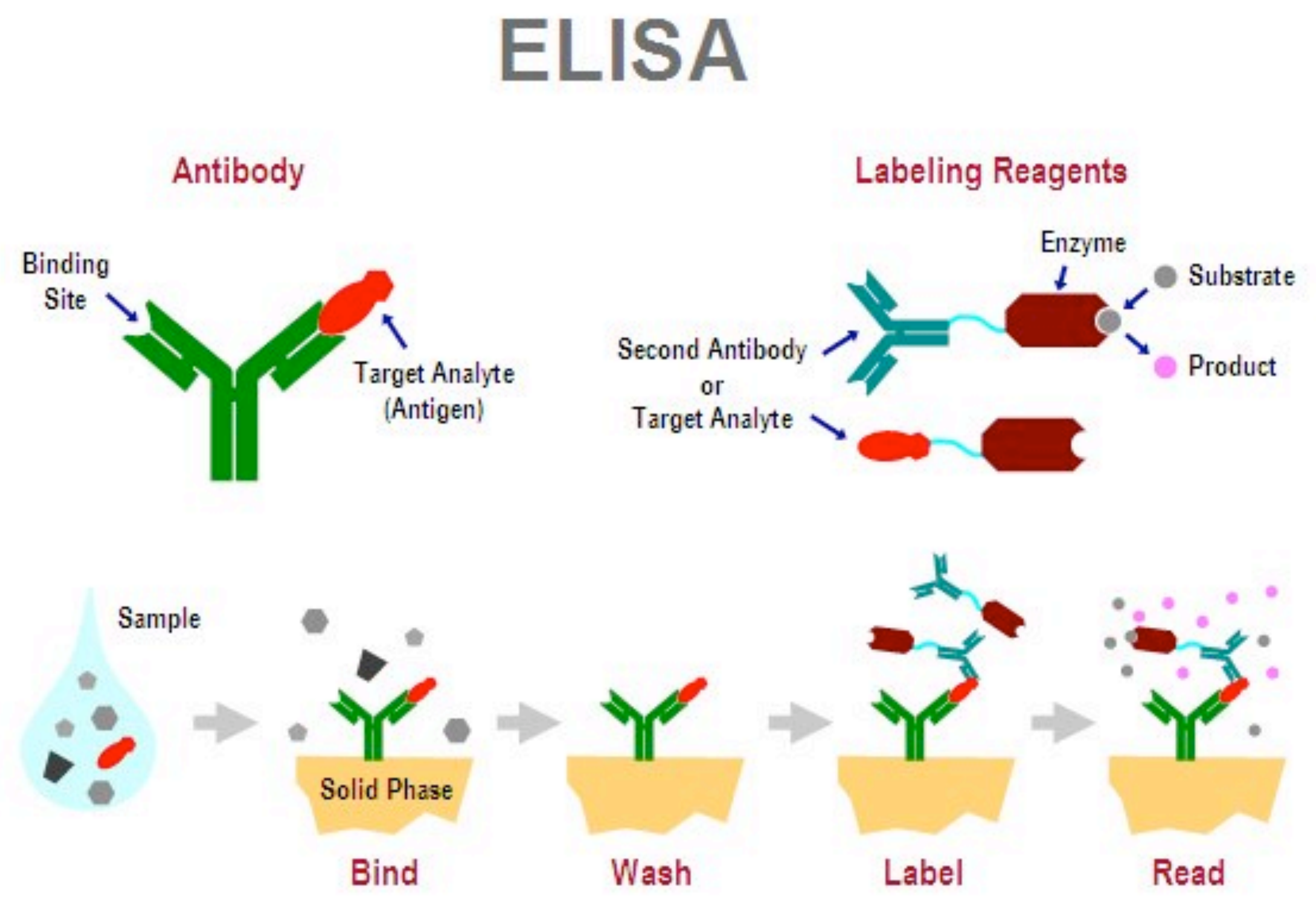






Other Outcomes of Blood Test: ELISA and Pathogens

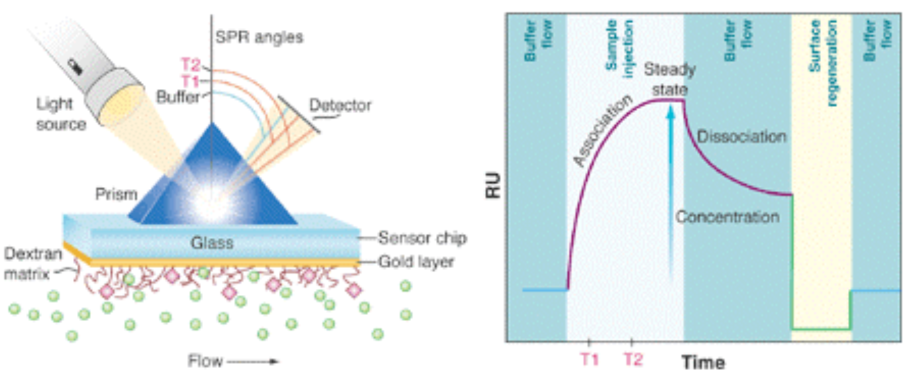
Enzyme-Linked ImmunoSorbant Ass



http://www.biosystemdevelopment.com/site graphics/elisa.jpg

How Are Measurements Made? What Is the Data Like?

Surface Plasmon Resonance



Wilson, W. Science 295, 2103 (2002)

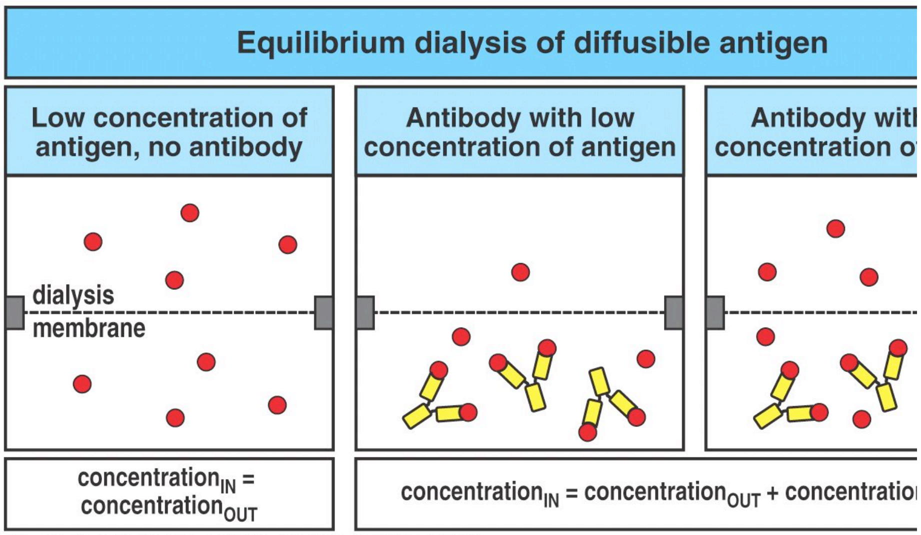
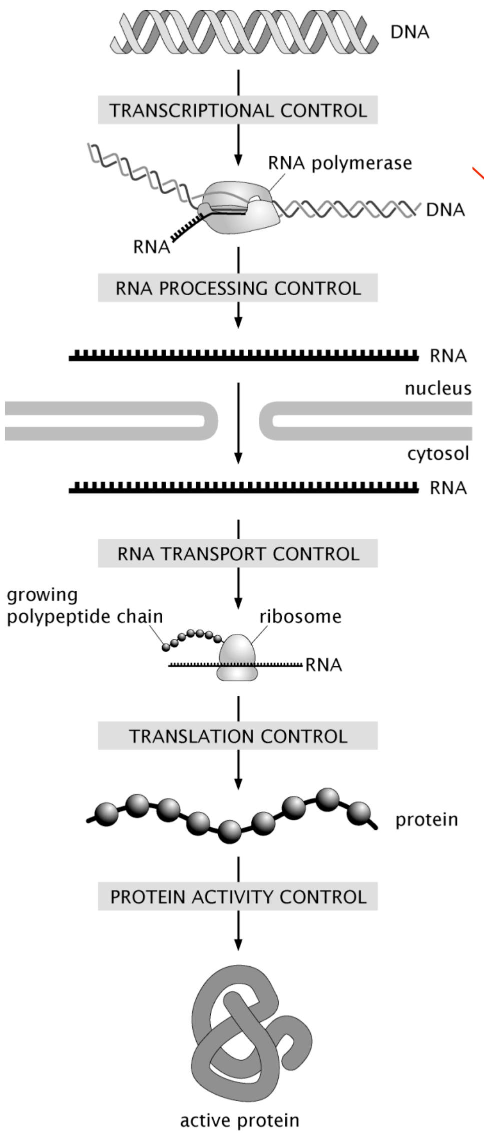


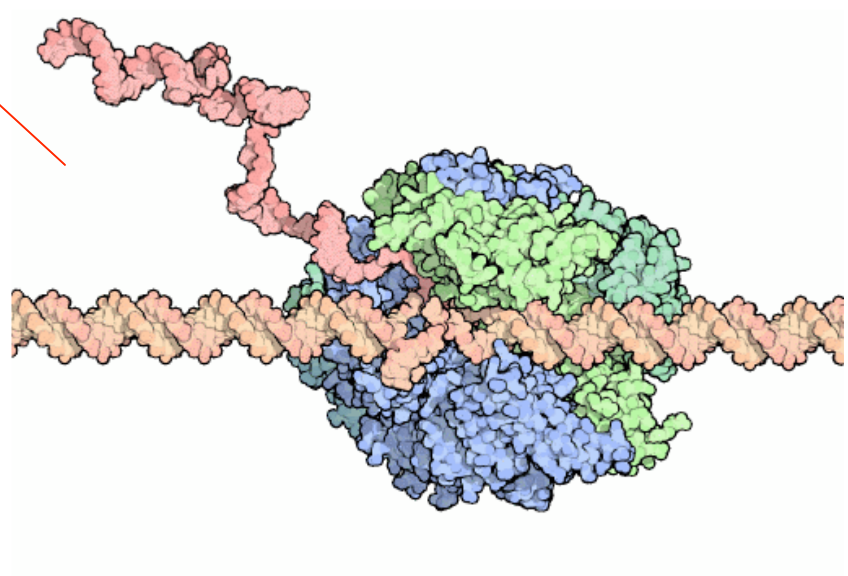
Figure A-11 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

- *More tricks for examining the extent of binding between molecular partners.*
- *Always ask yourselves, how do they know the mechanism? How do they know the numbers?*

How Cells Decide: Transcriptional Regulation

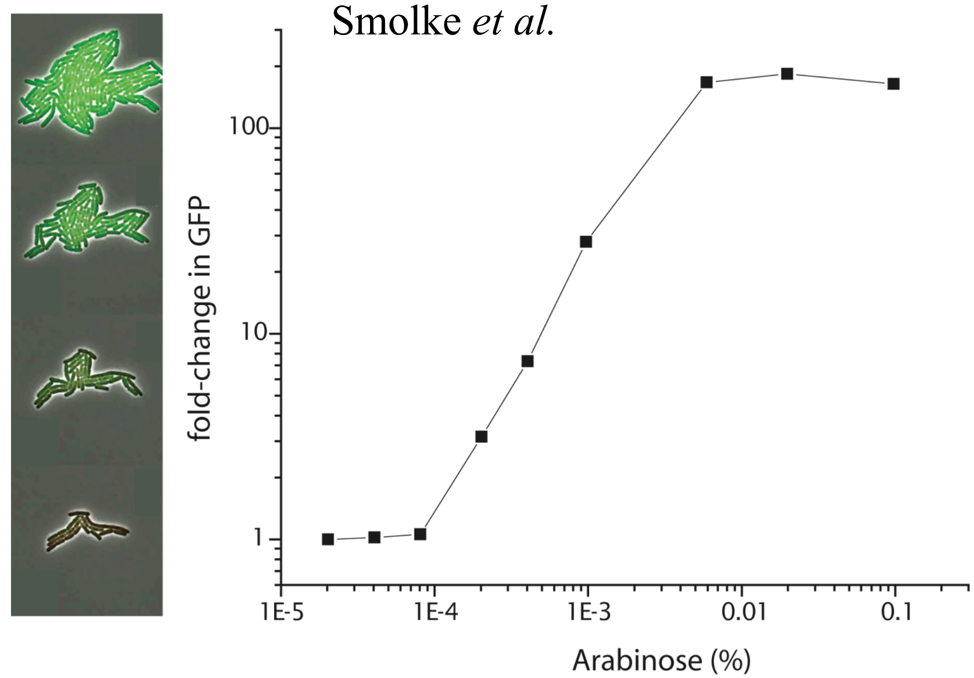


- ***Regulation takes place very far upstream. In particular, the "decision" is made whether o not to produce mRNA.***
- ***Question: What are the molecules that medi this control?***
- ***Molecular binding events on DNA are a key mechanism of control.***

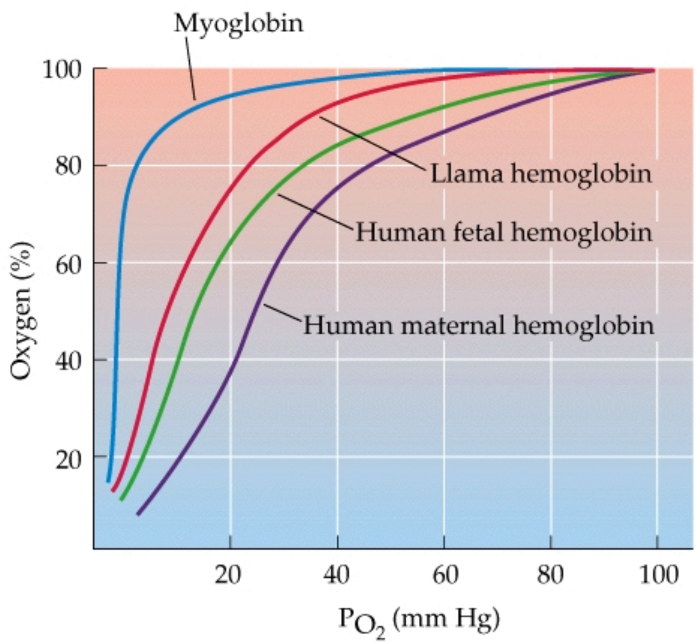


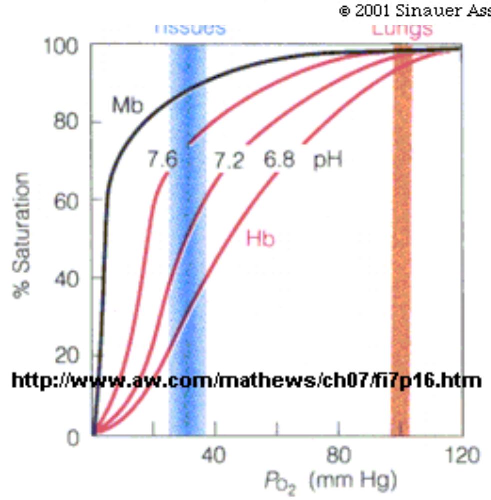
How Are Measurements Made? What Is the Data Like?

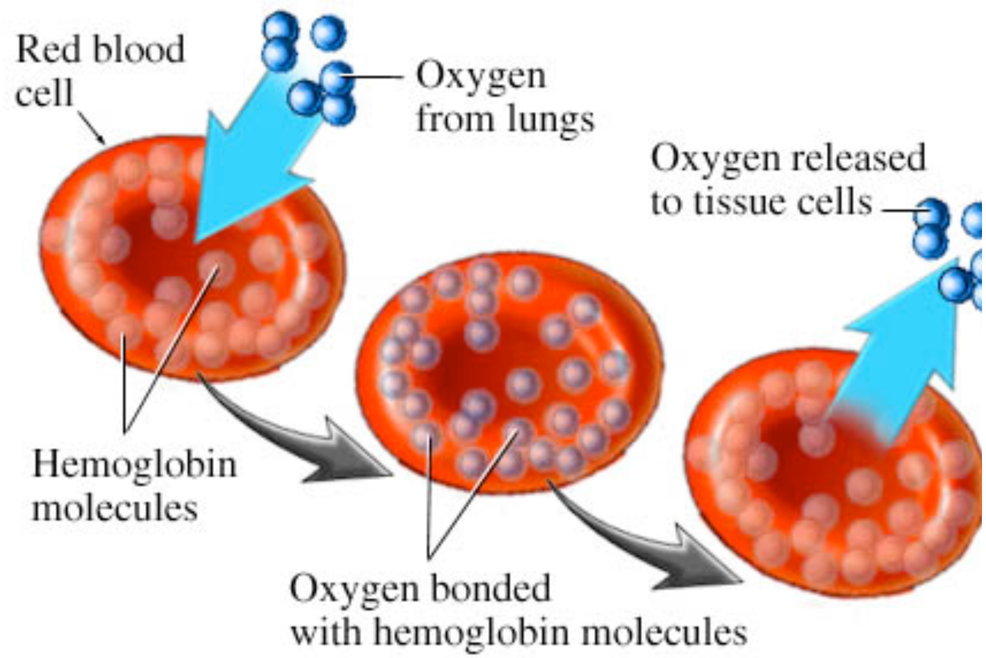
- ***Measure the relative change in the amount of a given gene product as a function of some tuning variable such as the concentration of a particular inducer.***
- ***Gene expression is the ``readout'' of underlying molecular binding events on DNA.***



Hemoglobin Binding Curves: Yet Another Example of Biologically-Important Binding



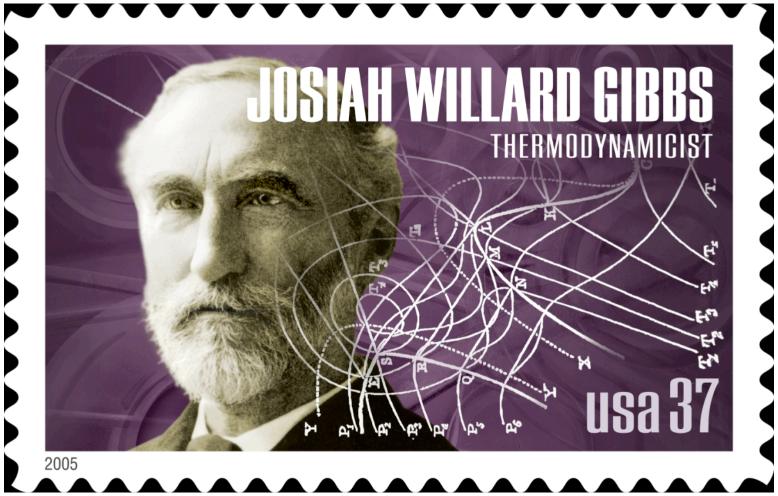




- *``Corpora non agunt nisi ligata'' - ``A substance not effective unless it is linked to another.'' - Pau Ehrlich*
- *O2 binding curves a great other example*

Unleashing the Tools of Statistical Mechanics

- *Our aim is to calculate binding curves.*
- *We will need certain very important tools to go back and forth between our theoretical analysis and the data: law of mass action, lattice model of solution, mathematics of balls and urns,…*



Ludwig Boltzmann

An Intuitive Description of Binding as a Battle Between Energy and Entropy

- *Free energy competition between energy and entropy.*
- *Binding probability as a ratio.*